APPLICATION NOTE

High-Throughput, End-to-End Cell Free DNA Analysis Workflow from Plasma

PerkinElmer's Automated cfDNA Workflow

Highlights

- Automated plasma collection from fractionated blood
- High quality cfDNA extraction from 96 samples
- Reliable, hands-free NGS library generation
- High-throughput library quality control

Introduction

The presence of double stranded, circulating cell free DNA (cfDNA) in blood plasma was discovered more than 70 years ago[1]. Soon after, a link between cfDNA and leukemia[2] as well as autoimmune disease[3] was found. Recently, the development of cfDNA-based prenatal genetic testing and the realization that cfDNA can be used to detect and monitor tumor specific mutations in cancer patients has increased the interest in cfDNA analyses[4].

cfDNA is an attractive substrate as it is amenable for inexpensive noninvasive testing and serial sampling. However, it also presents technical challenges such as low concentrations and high levels of fragmentation – in plasma and serum, cfDNA derived from apoptosis is present as fragments of approximately 167 bp and multiples of this number in healthy individuals, 150 bp and multiples of this number in cancer patients[5]. Longer DNA fragments might be present as well due to contamination from ruptured blood cells.

As indicated in previous notes[6,7], reliable automated solutions for plasma isolation, cfDNA extraction and processing are critical for sensitive downstream applications such as next-generation sequencing (NGS). In this work, we continue to illustrate the benefits of the completely automated workflow developed by PerkinElmer for high-throughput cfDNA analysis.

Method

Plasma sample preparation

Blood from 96 healthy donors (9 mL) were collected in K2EDTA tubes. Donors 1-48 were collected in Vacuette® tubes while Donors 49-96 were collected in Vacutainer® tubes. Plasma was separated from RBCs and WBC with the JANUS® G3 Blood iQ™ Workstation. Output plasma volume was 4.1-4.5 mL. No correlation was observed between tube type and the volume of plasma obtained.

cfDNA Extraction

1.5 mL of plasma from each of the 96 donors (1 replicate/donor) was used as input on the chemagic™ 360 instrument equipped with a 96-rod head. cfDNA was purified using the chemagic™ cfDNA 1.5k Kit H96 (CMG-1396). A JANUS® G3 Primary Sample Reformatter was used to set up all plates for the chemagic™ 360 instrument. Purified samples were quantified with the Thermo Scientific® Qubit® assay.

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Library Preparation

An equal volume of extracted cfDNA (50 µL) was transferred to a 96-well hard-shelled PCR plate and used as input for whole genome library preparation on the Sciclone® G3 NGSx workstation. Libraries were made using the NEXTFLEX® Cell Free DNA-Seq Library Prep Kit 2.0, with the option of gel-free nucleosome enrichment. NEXTFLEX® Unique Dual Index Barcodes were diluted 1:8. Libraries were quantified using the NGS 3K assay on the LabChip® GX Touch™ HT nucleic acid and the Thermo Scientific® Qubit® assay.

Results

The isolation of cfDNA using the chemagic cfDNA 1.5k Kit on the chemagic™ 360 instrument shows a highly reproducible yield, with an average of 13.08 ng of cfDNA recovered/mL plasma and CV=24.15% (Figure 1). These results are in line with the amounts of cfDNA reported in the literature for healthy subjects.

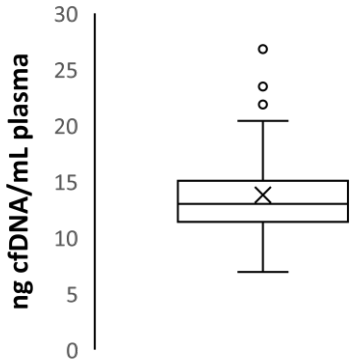


Figure 1. *Highly reproducible cfDNA extractions on chemagic™ 360 instrument. 1.5 mL of plasma from healthy donors was obtained with the JANUS® G3 Blood iQ™ Workstation and cfDNA was extracted in one automated run.*

The subsequent cfDNA eluates were used to construct libraries for whole genome sequencing. Average yield was 113.91 ng/µL as measured with Qubit® and as expected, a tight correlation was found between the input amount used and the yield obtained.

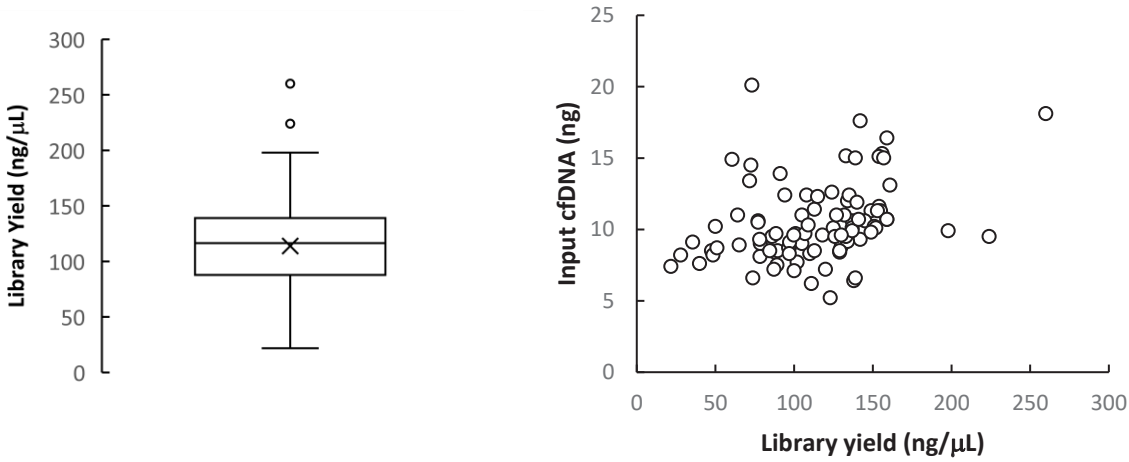


Figure 2. *Yields obtained from libraries prepared in a single automated run on the Sciclone® G3 NGSx workstation. Same volume of extracted cfDNA were used as input of the libraries. Differences in library yield correlate closely with the amount of cfDNA introduced as template.*

Analysis of the size of the libraries obtained show a bimodal distribution, with about 90% of the total distribution contained in low molecular weight band (~300 bp corresponding to mononucleosomal cfDNA). The second peak is compatible with the dinucleosomal fragment size (Figure 3).

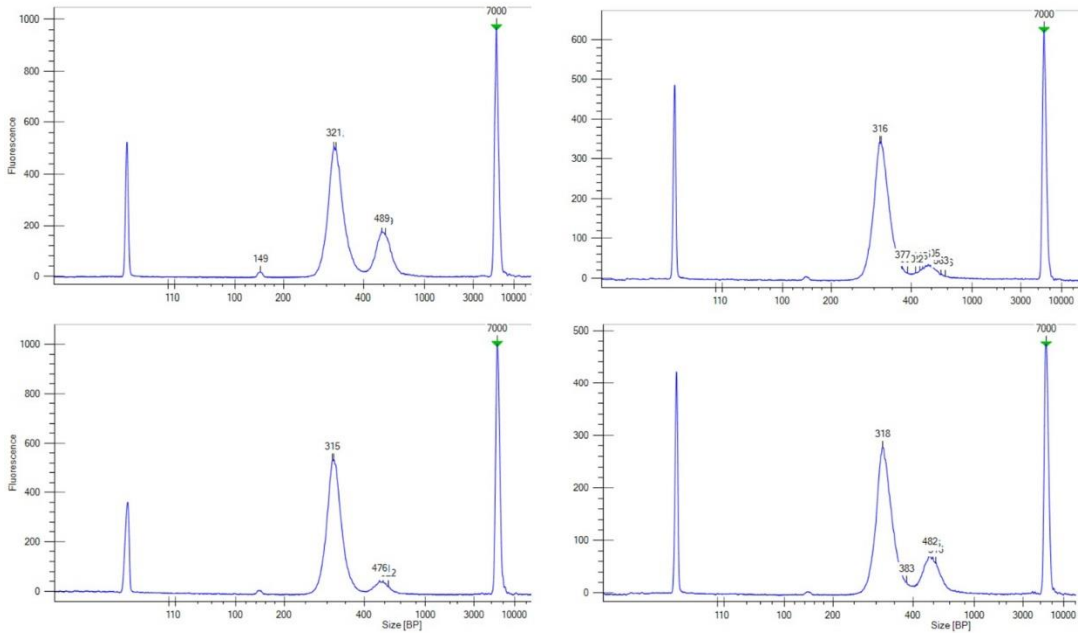


Figure 3. *Example of final libraries ready for sequencing as appearing on the LabChip® GX Touch™ HT nucleic acid analyzer.*

Conclusion

We present here data illustrating the reliability and reproducibility of an automated workflow covering all the steps in the process, from plasma extraction to library preparation. By incorporation of these solutions, labs can increase their operational capacities, produce libraries of consistent high-quality for NGS and minimize risk of errors.

Contact us to explore the advantages of a reliable, automated cfDNA workflow

AUTOMATED PLASMA COLLECTION FROM FRACTIONATED BLOOD

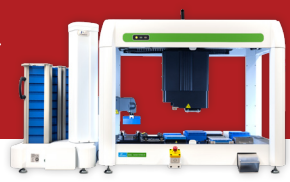
Blood Fractionation:
JANUS® G3 Blood iQ™ Workstation

HIGH QUALITY cfDNA ISOLATION FROM PLASMA

Nucleic Acid Extraction:
chemagic™ 360 Instrument

Cell-Free DNA Extraction:
chemagic™ cfDNA 1.5k Kit H96

WALK-AWAY AUTOMATION & RELIABLE CHEMISTRIES FOR LIBRARY PREP



NGS Library Preparation:
Sciclone® G3 NGSx iQ™ Workstation

cfDNA Library Preparation:
NEXTFLEX® Cell free DNA-Seq Kit 2.0

HIGH-THROUGHPUT QC & QUANTITATION OF LIBRARIES

Library Quality Control:
LabChip® GX-Touch™ Microfluidic Technology

References

1. Mandel P. Les acides nucleiques du plasma sanguin chez 1 homme. CR Seances Soc. Biol. Fil. 1948;142:241–243.
2. Tan E.M., Schur P.H., Carr R.I., Kunkel H.G. Deoxybonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. J. Clin. Invest. 1966;45:1732–1740.
3. Koffler D., Agnello V., Winchester R., Kunkel H.G. The occurrence of single-stranded DNA in the serum of patients with systemic lupus erythematosus and other diseases. J. Clin. Invest. 1973; 52, 198–204.
4. Stanislav Volik, Miguel Alcaide, Ryan D. Morin and Colin Collins. Cell-free DNA (cfDNA): Clinical Significance and Utility in Cancer Shaped By Emerging Technologies. Mol Cancer Res October 1 2016; (14) (10) 898-908; DOI: 10.1158/1541-7786.MCR-16-0044.
5. Underhill H.R. Leveraging the Fragment Length of Circulating Tumour DNA to Improve Molecular Profiling of Solid Tumour Malignancies with Next-Generation Sequencing: A Pathway to Advanced Non-invasive Diagnostics in Precision Oncology? Molecular Diagnosis and Therapy 2021; 25(4), 389–408; DOI: 10.1007/S40291-021-00534-6/TABLES/4.
6. Application Note: A start-to-finish cfDNA extraction and sequencing automation experience from plasma/serum.
7. Technical Note: Automated Circulating Cell-Free DNA Purification with the chemagic™ 360 Instrument.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

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